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Effects of duration of early feed withdrawal and re-feeding on growth, carcass traits, plasma constituents and intestinal microflora of broiler chickens

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ABSTRACT

The effect of different feed withdrawal durations and re-feeding on broiler performance was investigated. A total of 225 broiler chicks (Ross 308) were assigned to 5 groups; control (full feeding) and feed withdrawal for 6, 8, 10 and 12 h daily. Feed intake and weight gain were reduced ($P < .05$) in the feed withdrawal groups during the starter phase, but were not affected during later growth. Feed conversion ratio was not affected by the treatment. The relative weight of carcass was reduced ($P < .05$) in 6 and 12 h feed withdrawal groups. Breast weight was lowest on the control group and highest on the 8 h withdrawal ($P < .05$) group. The weight of the thigh was reduced ($P < .05$) on the control group. Plasma protein was higher on the control group and 6 h group compared to 10 h feed withdrawal group ($P < .05$). Plasma albumin was reduced on 12 and uric acid on the control and 6 withdrawal groups. There were no treatment effects on plasma cholesterol, HDL and LDL values. *E. coli* count was not affected by treatment, but Lactobacilli count was higher ($P < .05$) on the 12 h feed withdrawal group. Young broilers subjected to feed withdrawal for 8–10 h daily can compensate for performance losses in the early stage of growth and maximize the relative weights of carcass, breast muscle and thigh, but withdrawal lengths of 6 and 12 h might be too short and too long, respectively, to improve performance losses after resumption of full feeding.

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Feed withdrawal; growth compensation; gut health; broiler chickens

1. Introduction

Breeding has been very successful in poultry with broilers attaining about 2 kg at 33 d of age (Wilson 2005). This fast growth, however, requires nutrients dense and highly digestible feeds. On *ad libitum* feeding, broilers may consume up to three times their maintenance requirements (Barbato 1994), become susceptible to various metabolic disorders (Olkowski et al. 2008; Kalmar et al. 2013; Wideman et al. 2013) and deposit more abdominal fat (Barbato 1994; Mushtaq et al. 2014). High abdominal fat deposition in broilers is not a desirable attribute to the consumer due to higher cooking loss. Feed restriction in the early life has been reported to reduce abdominal fat deposition (Plavnik and Hurwitz 1985; Plavnik et al. 1986; Molapo and Webb 2014) through delayed hyperplasia and/or hypertrophy of adipocytes (Plavnik and Hurwitz 1985; Plavnik et al. 1986). However, more research is needed in this subject as factors including the method of restriction, age of birds during restriction and composition of the post restriction diet may all affect performance results. The present study investigated the effects of different lengths of feed withdrawal during the second week of age (8–14 d) on the growth, carcass traits, plasma constituents and intestinal microflora of broiler chickens. It was hypothesized that feed withdrawal during the early age and re-feeding will improve broiler growth, meat quality and gut health.

2. Materials and methods

2.1. Experimental diets

Broiler diets were formulated at three phases of growth (starter: 1–14 d; grower: 15–28 d and finisher: 29–42 d) to meet the nutritional requirements based on Ross strain rearing catalogue (Aviagen 2014, p. 8). The ingredients and nutrient compositions of the diets are presented in Table 1.

2.2. Experimental birds and management

Two hundred and twenty five male Ross 308 day-old chicks were randomly assigned to 15 cages measuring $1 \times 1 \times 2$ m. During the first week, all birds were fed *ad libitum*. From 8 to 14 d, birds in three replicate pens were subjected to one of following feeding regimens/treatments; control (full feeding), and withdrawal for 6, 8, 10 and 12 h in a completely randomized design. The building temperature and relative humidity were maintained between 30°C and 33°C and 55–65%, respectively, by gasoline heaters and spraying water on the floor during the experimental period. The lighting programme consisted of 24 h on the first day and 23 h from the second day to the end of the experiment.

All chicks were placed on *ad libitum* feeding immediately after the withdrawal period. Water was provided *ad libitum* throughout the experimental period. The experimental protocol

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Table 1. Ingredients' composition and nutrient analysis of experimental starter (1–14 d), grower (15–28 d) and finisher (29–42 d) diets.

Ingredients (g/kg)	1–14 days	15–28 days	29–42 days
Corn	475.5	567	589
Soybean meal	390	330	315
Wheat	85	55	50
Soybean oil	2	5	5
Di Calcium Phosphate	19	17	16
Calcium carbonate	13.5	12.2	12
DL-Methionine	2.7	1.7	1.3
NaCl	4.2	4.2	4.2
Lysine-Hydro-Chloride	3.1	2.9	2.5
Vitamin[a] and Mineral premix	5	5	5
Analysed composition			
Crude protein (%)	22.85	19.85	19
Calculated composition			
ME (Kcal/kg)	2880	3050	3050
Calcium (%)	1.05	0.9	0.85
Available phosphorus (%)	0.5	0.45	0.42
Sodium (%)	0.18	0.18	0.18
Methionine (%)	0.66	0.55	0.49
Lysine (%)	1.43	1.24	1.09
Methionine + Cysteine (%)	1.07	0.95	0.86

[a]Bio-mix supplied/kg diet vitamin A, 10,000 mg; D3, 2,000 mg; vitamin E, 23 mg; vitamin B1, 1.8 mg; vitamin B2, 5 mg; vitamin B6, 3 mg; vitamin B12, 0.015 mg; vitamin K3, 2 mg; pantothenic acid, 7.5 mg; vitamin Biotin, 0.06 mg; and choline chloride, 300 mg.
[b]Mn, 40 mg; Fe, 20 mg; Zn, 30 mg; Cu, 3 mg; I, 0.44 mg and Se, 0.2 mg; antioxidant 1.25 mg.

was approved by the Rasht Branch, Islamic Azad University Ethic Committee, and care was taken to minimize stress on the birds.

2.3. Measurements

2.3.1. Growth and carcass data

Feed consumption and growth were recorded per pen weekly and feed conversion ratio (FCR) was calculated as feed consumed to weight gained. On day 42, 2 birds per pen were slaughtered by decapitation for carcass and visceral segment measurements. Slaughtered birds were scalded at 50°C for about 1 m, plucked, eviscerated and dressed. The dressed chickens and carcass cuts (breast and thigh), gizzard, liver and abdominal fat pad were weighed and expressed as percentages of the live weight.

2.3.2. Haematological traits

At the end of the experimental period (day 42), blood samples were collected from 1 bird per replicate. From the wing vein, blood samples (1 ml/bird) were collected into sample tubes containing EDTA for haematological analysis. After centrifugation (3000 *g* for 10 min at room temperature), the plasma was harvested into eppendorf tubes and stored at −20°C until analysis. Biochemical analysis was performed using standard protocols of commercial laboratory kits (Pars Azmoon Co., Tehran, Iran) following the manufacturer's instructions. Glucose was measured by a glucose-oxidase photometric assay. Cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were determined by enzymatic CHOD-PAP (Tan et al. 1991). Uric acid was determined by enzymatic methods using the uricase-TOOS method (Kato et al. 2000). Albumin was determined based on the bromocresol green method (Maxwell et al. 1990) and total protein assayed by the Biuret method (Maxwell et al. 1990). Globulin was calculated by the difference between the total protein and albumin. Alkaline phosphatase was assayed by the method described by Thomas (1998).

2.3.3. Intestinal microflora

After slaughter, the ileum was removed and immediately placed in culture-specific environments. Agar plates were streaked with colon contents and sent to the laboratory. To determine bacterial growth and colony counts, the agar collecting tubes were weighed, wrapped in an aluminium sheet and autoclaved for 10 min. The culture media were prepared 24 h before samples were poured into petri dishes. de Man, Rogosa, Sharpe agar was used to culture *Lactobacilli*, and eosin methylene blue was used to culture *Escherichia coli*. Samples were transferred to the laboratory in the listed tubes and weighed again and the amount of sample in each tube was calculated by the difference between these two values. Tubes were shaken for approximately 30 min for the isolation of bacteria from gastrointestinal contents and preparation of suspension. This was done by adding 9 ml of PBS to 1 ml of the prepared suspension in the other tube. The suspension was prepared from 10^{-1} dilutions and serial dilutions were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). About 100 µl was removed from dilutions (10^{-4}, 10^{-5} and 10^{-6}) and poured into the petri dish previously prepared containing the medium and uniformly distributed to all parts of the medium. *Lactobacilli* were incubated at 37°C in

Table 2. Effect of duration of feed withdrawal on growth performance of broilers.

		Feed withdrawal groups					
Parameters	Control	6 h	8 h	10 h	12 h	SEM	*P*-value
Feed intake (kg)							
8–14 days	517.7[a]	462.6[b]	459.5[b]	456.1[b]	459.6[b]	10.442	0.036
15–28 days	2719.7	2746.9	2745.9	2735.3	2750.3	4.978	0.187
29–42 days	1155.9	1167.9	1173.5	1173	1176	2.782	0.132
8–42 days	4393.2	4377.4	4378.9	4363.4	4385.9	4.429	0.098
Weight gain							
8–14 days	435.8[a]	391.9[b]	390.3[b]	387.4[b]	389.5[b]	8.246	0.023
15–28 days	1403	1405.1	1404.6	1402.4	1405.9	0.589	0.241
29–42 days	502.3	505.1	503.7	501.3	506.7	0.612	0.199
8–42 days	2341.5	2305.4	2298.5	2291.1	2300	16.874	0.0672
FCR (feed: gain)							
8–14 days	1.18	1.18	1.17	1.17	1.18	0.132	0.065
15-–28 days	1.93	1.95	1.95	1.95	1.96	0.252	0.061
29–42 days	2.30	2.32	2.33	2.34	2.33	0.254	0.082
8–42 days	1.87	1.89	1.90	1.90	1.90	0.157	0.085

Note: Means in the same row with different subscripts differ significantly ($P < .05$).

anaerobic conditions for 72 h. An anaerobic jar was used to create anaerobic conditions. Counting of bacteria in the petri dishes was done by a colony counter. Bacterial counts were reported as logarithm number of bacteria per g sample.

2.4. Statistical analysis

Data were subjected to one-way analysis of variance of a completely randomized design using the General Linear Model procedures of SPSS (1997). Treatment means were compared using the Duncan Multiple Range Test at $P \leq .05$.

3. Results

3.1. Growth performance

During the starter phase (8–14 d), feed intake was significantly reduced ($P < .05$) in the feed withdrawal groups compared to the control groups. Feed intake did not differ among the withdrawal groups. There was no treatment effect on feed intake during the grower, finisher and overall growth periods. Body weight gain followed a similar pattern to feed intake. Feed conversion ratio was not affected by treatment during any of the growth phases (Table 2).

3.2. Carcass and organ measurements

The results of carcass and organ measurements are presented in Table 3. The relative weight of carcass was reduced ($P < .05$) on the groups subjected to 6 and 12 h feed withdrawal compared to the control and 8 h withdrawal groups. The lowest weight of the breast was recorded on the control and the highest value on 8 h withdrawal groups ($P < .05$). Breast meat weight did not differ between 8 and 10 h and among 6, 10 and 12 h feed withdrawal. The weight of the thigh was markedly reduced ($P < .05$) on the control compared to the feed withdrawal groups. A significantly lower liver weight was recorded on the control compared to the 8, 10 and 12 h feed withdrawal groups. There were no significant differences in relative weights of the thigh and liver among the feed withdrawal groups. There were no significant treatment effects on the weights of the gizzard and abdominal fat.

Table 3. Effect of early feed withdrawal and re-feeding on carcass some traits of broilers.

	Relative weight of carcass and organs (% live weight)					
Treatments	Carcass	Breast	Thigh	Liver and bile	Gizzard	Abdominal fat
Control	67^{a}	15.5^{c}	15.9^{b}	1.8^{b}	2.2	0.5
6 h withdrawal	62^{b}	22.7^{b}	19.5^{a}	2.2^{ab}	2.3	0.5
8 h withdrawal	67.3^{a}	24.9^{a}	21.4^{a}	2.4^{a}	2.6	0.4
10 h withdrawal	66^{ab}	23^{ab}	21^{a}	2.5^{a}	2.3	0.5
12 h withdrawal	63^{b}	22.9^{b}	21.6^{a}	2.4^{a}	2.6	0.5
SEM	0.964	1.451	1.442	0.322	0.121	0.018
P-value	0.022	0.014	0.011	0.006	0.008	0.001

Note: Means with different subscripts in the same column differ significantly ($P < .05$).

3.3. Plasma constituents and intestinal microflora count

Plasma protein concentration (Table 4) was higher on the control and 6 h than 10 h feed withdrawal ($P < .05$) group. The lowest value of albumin was recorded on the 12 h withdrawal group. Plasma concentration of uric acid was lowest on the control and 6 h feed withdrawal group. There were no treatment effects on the plasma cholesterol, HDL and LDL values.

Results of intestinal microflora count (Table 5) showed no significant treatment effect on the count of *E. coli*. Lactobacilli count was significantly ($P < .05$) higher on the 12 h feed withdrawal group, but did not differ between the other withdrawal groups and the control group.

4. Discussion

4.1. Growth performance

The lower feed intake and body weight gain on the withdrawal groups during the starter phase and similarities in overall feed intake and weight gain among all the treatments suggest a compensation of lost performance during the period of feed withdrawal. Jang et al. (2009) also reported compensatory growth in early-restricted broiler chickens grown only over a 35 d period. Contrary to these findings however, Ramlal et al. (1996) did not obtain full compensation in the growth of early feed restricted broilers during a 42 d growth period. Several factors, including the severity and timing of feed restriction, length of time allowed for re-feeding and strain of birds, have all been reported to affect weight compensation in restricted fed broilers (Yu and Robinson 1992). The enhanced growth

Table 5. Effect of feed restriction on intestinal microflora of broilers (log_{10} cfu/g).

Treatments	*E. coli*	*Lactobacilli*
Control	8.6	8.9^{b}
6 h withdrawal	8.3	8.9^{b}
8 h withdrawal	8.0	8^{b}
10 h withdrawal	7.9	8.8^{b}
12 h withdrawal	8.0	9.5^{a}
SEM	0.986	2.63
p-Value	0.277	0.021

Note: Means with different subscripts in the same column differ significantly ($P < .05$).

Table 4. Effect of feed withdrawal on plasma constitutes (mg/dl) of broilers.

Treatments	Total protein	Albumin	Globulin	Cholesterol	Triglycerides	HDL	LDL	Uric acid
Control	4.46^{a}	3.28^{ab}	161.33	139.67	54	60	68.76	4.31^{b}
6 h withdrawal	4.43^{a}	2.85^{bc}	165	120.67	62.66	62.33	45.8	4.43^{b}
8 h withdrawal	3.93^{ab}	3.56^{a}	163	112.33	49.33	57.66	44.8	5.66^{a}
10 h withdrawal	3.76^{b}	2.91^{bc}	166.33	116.67	42.66	61.66	46.47	5.85^{a}
12 h withdrawal	4.02^{ab}	2.73^{c}	159.33	130.37	41.66	73.66	46.47	5.58^{a}
SEM	0.150	0.159	6.016	10.556	7.039	5.923	11.850	0.236
p-Value	0.043	0.022	0.923	0.407	0.270	0.411	0.591	0.002

Note: Means with different subscripts in the same column differ significantly ($P < .05$).

performance of modern broiler strains (Chang 2016) and improved feed utilization through better structure and function of several digestive organs (Mussini 2012) may explain the performance compensation during 42 d growth period in the present study.

4.2. Carcass and organ measurements

The effect of feed restriction on carcass traits has been variable. Some authors (Tumova et al. 2002; Jahanpour et al. 2015) reported increased carcass weight in feed-restricted broilers, while others (Summers et al. 1990) observed no statistical differences in carcass weight of young broilers subjected to feed restriction and re-feeding. The reason for reduced carcass weight in the 6 and 12 h feed withdrawal groups in this study was not clear, but the reasons may be too short and too prolonged periods of feed deprivation, respectively. The lower relative weight of breast muscle on the control group compared to the withdrawal groups is in agreement with the earlier report by Tumova et al. (2002) who observed an increase in relative weight of breast muscle in feed-restricted broilers following re-alimentation. Like that of the breast, the relative weight of the thigh was also reduced in the control group suggesting that when we give access to feed after a period of nutritional stress, broilers may shift their metabolic pathway and tissue synthesis. Similar to our findings, Fontana et al. (1993) also observed no statistical effect of feed restriction on gizzard weight of broiler chickens. The higher liver weight in the feed withdrawal groups in this study may be attributed to the

overconsumption of feed in these birds thus increasing liver activity. This pattern of liver weight agrees with the reports of Zubair and Leeson (1996) and Ozdogan and Aksit (2003) who observed a significant increase in the liver weight of feed-restricted and re-alimented broilers. Reduced abdominal fat in early restricted birds has been attributed to the reduced capacity of the liver to produce fat during feed restriction (Jones and Farrell 1992) probably due to the dystrophy of this organ during fasting. In the present study, however, liver weight was increased in the feed withdrawal groups probably as a result of increased feed consumption after the period of fasting. This may explain the similarity in abdominal fat among the treatments groups.

4.3. Plasma constituents and intestinal microflora count

The pattern of plasma constituents in this study could not be explained, but it may be probably due to a shift in metabolic pathways occasioned by nutritional stress as suggested earlier. The alkaline pancreatic juice plays an important role in the maintenance of intestinal pH. Reduced pancreas mass has been reported in fasted birds (Chediack et al. 2012). Although pancreas weight was not monitored in the present study, insufficient production of pancreatic juice, favouring a higher intestinal pH in the 12 h withdrawal group could be speculated as a possible factor for the higher *Lactobacilli* count in this group. Environments which favour beneficial bacteria normally suppress the count of pathogenic bacteria. The reason for higher *E. coli* count on the 12 h withdrawal group despite the higher *Lactobacilli* count in this study was not understood and needs more research.

5. Conclusions

Young broilers subjected to feed withdrawal for 8 and 10 h daily during the second week (8–14 d) can compensate for performance losses in the early stage of growth and maximize the relative weights of carcass, breast muscle and thigh. Withdrawal lengths of 6 and 12 h might, however, be too short and too long, respectively, to improve performance after resumption of full feeding. More research in the age, period of the day during withdrawal and the composition of the diets before and after withdrawal is recommended.

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Disclosure statement

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